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September 26, 2005 Our Ref.: 050926-308
Via E-Mail and Hand Delivery

Mr. R. Lance Wormell
Special Review and Reregistration Division (MC 7508C)
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 South Bell Street
Arlington, VA 22202

Re: Comments on *Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation*

Dear Mr. Wormell,

The Methanearsonic Acid (MAA) Research Task Force (Task Force) herewith submits comments on the U.S. Environmental Protection Agency (EPA) Health Effects Division (HED) preliminary draft document *Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation,* issued on July 26, 2005.

The Task Force appreciates the time and effort EPA has devoted to preparing this document, which contains a comprehensive evaluation of the mode of action (MOA) of cacodylic acid and provides a methodology for calculating a cancer reference dose for DMA^V. While we support EPA's use of a nonlinear approach to characterize DMA^V risk, there are a few specific aspects of the analysis which should be corrected before the final document is issued.

In the attached document, which was prepared together with Gradient Corporation, specific issues in EPA's analysis that warrant further considerations are identified, and in certain instances, a revised interpretation of the data is provided. We urge EPA to re-examine the issues discussed in the attached document and refine its analysis to reflect the most scientifically supportable approach.

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We hope that these comments will facilitate achieving the goal of a scientifically sound and accurate document.

Please do not hesitate to contact me should you have any questions.

Sincerely,

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MAATF

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Comments on "*Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation*"

This document contains comments on the preliminary draft "Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation," which was issued by the United States Environmental Protection Agency (US EPA, EPA) Health Effects Division (HED) on July 26, 2005 (US EPA, 2005a). EPA's Science Issue Paper contains a comprehensive evaluation of the mode of action (MOA) of cacodylic acid and provides a methodology for calculating a cancer reference dose for DMA^V. While we agree with EPA's use of a nonlinear approach for the characterization of DMA^V risk, we have identified specific issues in EPA's analysis that warrant further considerations and, in certain instances, provide a revised interpretation of the data. The following are our comments:

1. Page 3, Preface, First paragraph, lines 17-19: "It is important to note that following pesticide applications of MMAv to citrus and/or cotton plants, residues measured in the fruit and plants are predominately DMA."

This statement is far from being accurate. The Methanearsonic Acid (MAA or MMA) Research Task Force (Task Force) conducted several laboratory metabolism and field residues trials. The results of these studies are summarized in Table 1. In cotton, in both laboratory and field studies, most of the residues found were MMA, and DMA residues were very low if any, except for one case where the residue was 0.24 ppm. In citrus laboratory studies, DMA residues appeared to be higher than MMA residue based on percentage, however, the absolute concentrations of both MMA and DMA are very low and the same order of magnitude. For example, lemon pulp was found to contain 0.03 ppm of MMA and 0.04 ppm DMA (*i.e.*, 61% of the total residue). It should be mentioned, that the Task Force recently proposed master labels eliminating application to bearing citrus. In conclusion, there are hardly any residues in the crops following MSMA application, and most of the residues are MMA rather than DMA. The Task Force respectfully requests that the above mentioned statement be revised before a final version of the Science Issue Paper is published.

Table 1: Summary of MSMA and DMA residue data from MSMA studies

Crop	Crop Part	Comments	Residues		Reference
			MMA (ppm)	DMA (ppm)	
Cotton	Seed	Laboratory metabolism study 96 DAT[1]	0.80 (54%)	0.09 (6%)	PTRL, 1992a
		23 trials of 2 MSMA applications	12 - <0.05 max 0.15 average 0.06	18 - <0.05 max 0.24 average 0.04	PTRL, 1995b
		23 trials of one MSMA application	13 - <0.05 max 0.15 average 0.05	20 - <0.05 max 0.13 average 0.03	
		6 trials, 1-2 applications: ×1, ×3 & ×5 rate	2 - <0.05 max 0.16 average 0.1	All <0.05	PTRL, 1995c
		2 trials, 2 applications:× 5 rate	0.13 & 0.16	Both <0.05	PTRL, 1995c
	Hulls	2 trials, 2 applications:× 5 rate	0.40 & 0.42	Both <0.05	
	Meal	2 trials, 2 applications:× 5 rate	<0.05 & 0.17	Both <0.05	
	Oil	2 trials, 2 applications:× 5 rate	Both <0.05	Both <0.05	
Lemon	Peel	Laboratory metabolism study 28 DAT[1]	0.17 (41%)	0.24 (55%)	PTRL, 1992b
	Pulp		0.03 (36%)	0.04 (61%)	
	Juice		0.05 (40%)	0.06 (52%)	
Lemons & limes	Whole fruit	7 trials: 3 applications	All <0.05	6-<0.05 1-0.08	PTRL, 1995a
Grapefruit	Whole fruit	8 trials: 3 applications	7-<0.05 1-0.07	All <0.05	
Orange	Whole fruit	12 trials: 3 applications	All <0.05	All <0.05	
	Misc.[2]	Application of label rate – 8 parts	All <0.05	6- <0.05 1-0.05 1-0.07	PTRL, 1995d
		Application of ×5 label rate – 8 parts	6- <0.05 2-0.09	6- <0.05 1-0.25 1-0.17	

1 DAT – Days after treatment

2 Parts sampled: washed fruit, unwashed fruit, juice, wet pulp, dry pulp, molasses and oil.

2. Page 51, Table 3.6: Summary of key precursor events and urinary bladder tumor formation in female F344 rats administered DMA^{V} in feed

Table 3.6 provides a summary of DMA^{V} dose-response for various cancer precursor endpoints and bladder tumor incidence. According to the footnotes to the table, the data are based on the research of Dr. Samuel Cohen. However, on September 12, 2005, Dr. Cohen presented a different interpretation of the data to the SAB Arsenic Review Panel meeting, which we understand is based on further, more recent studies (Cohen, 2005). We believe that Dr. Cohen will submit the new data to EPA and request that the new information will be considered.

3. Page 75-87, Section 5.D.2: Benchmark dose analysis

To calculate benchmark doses (BMDs), the EPA used urothelial cytotoxicity and cell proliferation data from Arnold *et al*. (1999), using Benchmark Dose Software (US EPA, 2001). Arnold *et al.* (1999) reported the exposure of rats to DMA^{V} in units of ppm in feed. To use these data for a BMD calculation, the units were converted from ppm in feed to the average dose (*i.e.*, mg/kg/d) over the entire 10 week study period. These average doses were correctly calculated for the modeling of the cytotoxicity data (*i.e.*, 0, 0.2, 1, 4, and 9.4 mg/kg/d for doses of 0, 2, 10, 40, and 100 ppm, respectively); however, for the modeling of cell proliferation, the doses were incorrectly calculated, resulting in erroneous doses of 0, 0.1, 0.7, 2.6, and 6.5 mg/kg/d, and incorrect estimates of the BMD_{10}[1] and $BMDL_{10}$[2] values. The correct BMD_{10} and $BMDL_{10}$ values are 0.92 and 0.43 mg/kg/d, respectively, and the correct estimates of BMD_1[3] and $BMDL_1$[4] are 0.75 and 0.10 mg/kg/d, respectively. Table 2 presents the values from the Science Issue Paper and the corrected values that should replace them.

Table 2: Calculated doses and resulting BMDs using data from Arnold *et al*., 1999

Dose		**2 ppm**	**10 ppm**	**40 ppm**	**100 ppm**	**BMD_{10}**	**$BMDL_{10}$**	**BMD_1**	**$BMDL_1$**
mg/kg/d	EPA's paper	0.1	0.7	2.6	6.5	0.65	0.29	0.54	0.07
	Correct dose*	0.2	1.0	4.0	9.4	0.92	0.43	0.75	0.1

* Calculated by Gradient Corporation.

[1] BMD_{10} is calculated using EPA's benchmark dose software and is the dose associated with a 10 % response. In this case it is the dose associated with a 10% increase in cell proliferation above baseline.
[2] $BMDL_{10}$, is the 95% lower bound on the BMD_{10} estimate.
[3] BMD_1 is calculated using EPA's benchmark dose software and is the dose associated with a 1 % response. In this case it is the dose associated with a 1% increase in cell proliferation above baseline.
[4] $BMDL_1$, is the 95% lower bound on the BMD_1 estimate.

The computer output from the BMD analysis is presented Appendix A. All of the inputs used to recalculate the benchmark dose were identical to EPA's analysis, except for the use of the corrected doses. Figures 1 and 2 depict the revised calculated BMDs.

Furthermore, as presented by Dr. Beck on the September 12, 2005 meeting of the SAB Arsenic Review Panel (Beck, 2005), and discussed in detail in Appendix B, we believe that for the purpose of establishing a cancer reference dose for DMA^V, a point of departure based on the $BMDL_{10}$ (as opposed to the $BMDL_1$) for cell proliferation is preferred because the $BMDL_{10}$ is associated with less uncertainty, while still being conservative.

Figure 1. Regenerative Proliferation at 10 weeks from Arnold *et al.* (1999). Doses are expressed as mg/kg/d and mean responses are BrdU labeling indices. BMD_{10} and $BMDL_{10}$ are in blue.

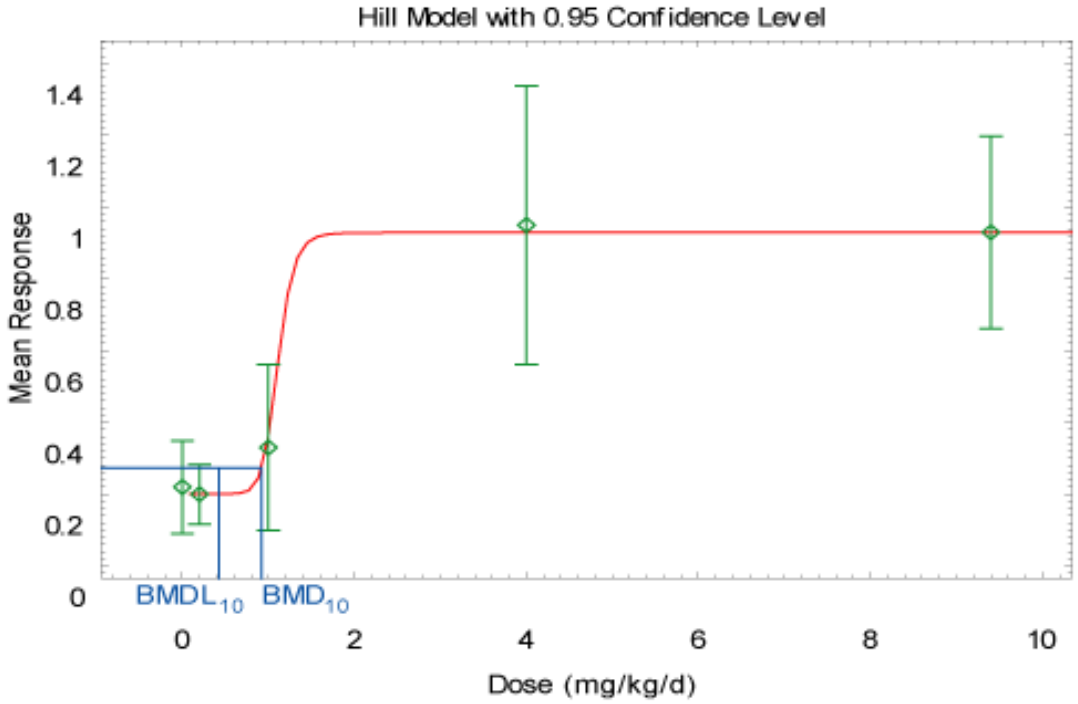
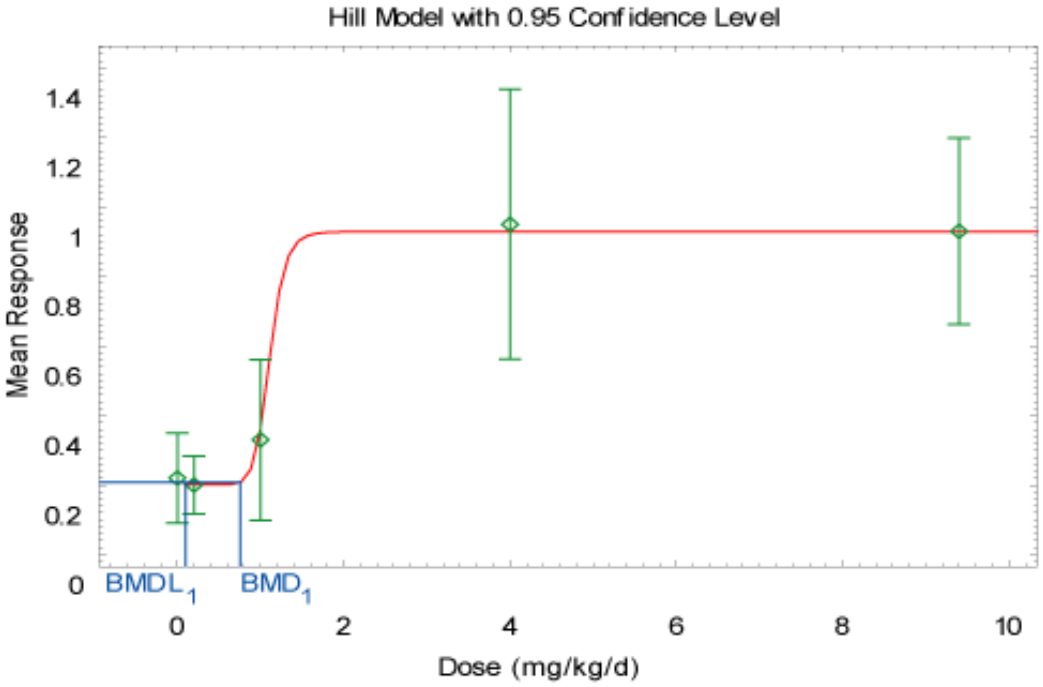


Figure 2. Regenerative Proliferation at 10 weeks from Arnold *et al.* (1999). Doses are expressed as mg/kg/d and mean responses are BrdU labeling indices. BMD_1 and $BMDL_1$ are in blue.



4. Page 91, Section 6: Summary and Conclusions – Interspecies differences

On page 91 of the Science Issue paper, EPA concludes that a 10X uncertainty factor (UF) should be applied to account for interspecies differences when developing the cancer reference dose for DMA. However, this conclusion is not supported by information that is discussed earlier in EPA's report, which presents a comprehensive review of the species-specific toxicokinetics and toxicodynamics of DMA, including evidence of rat-human differences. As the EPA report correctly notes, there "are important quantitative differences between these two species [that] need to be address and characterized in the risk assessment (p.35)."

Toxicodynamics

As discussed in more detail in Appendix B, there is no evidence that, based on toxicodynamic considerations, the human is more sensitive than the rat. DMA^{III} is cytotoxic in human and rat bladder cells at similar concentrations *in vitro* (Cohen *et al*., 2002). Additionally, using a microarray analysis, a group of researchers has presented preliminary results that suggest rat bladder cells may be more sensitive to gene perturbation in response to DMA^{V} compared to human cells (Sen *et al*., 2005). Because DMA affects human and rat bladder cells in a similar manner, with some evidence of human cells being less sensitive, we believe that a UF of 1 is appropriate to account for toxicodynamic differences.

Toxicokinetics

From information presented in EPA's report (and published in the scientific literature), it is well established that the toxicokinetics of DMA^{V} in rats differ from that in other species, including humans, in a number of respects. These differences make the rat uniquely susceptible to DMA^{V}-induced bladder tumors. Following DMA^{V} exposure, rats metabolize DMA^{V} to TMAO more than other species, generating relatively high levels of dimethylarsinous acid (DMA^{III}) as a metabolic intermediate. Yoshida *et al*. (1997) demonstrated that following administration of a single dose of radiolabeled DMA^{V} to rats, TMAO accounted for over 50% of all arsenic excreted between 6 and 24 hours after administration. Similarly, Yoshida *et al*. (1998) demonstrated that one week after exposure to DMA^{V} (100 mg As/L) in drinking water, 44.9% of all the arsenic excreted was eliminated as unchanged DMA^{V}, with 40% and 0.4% excreted in the form of further methylated metabolites TMAO and TMA, respectively. These findings are relevant to the MOA of rat bladder carcinogenicity from DMA. Specifically, generation of DMA^{III} from metabolism of DMA, to the extent that DMA^{III} is present at sufficient concentrations to induce a compensatory proliferative response, has been correctly identified by EPA as the key rate-limiting step in formation of the rat bladder tumors.

In contrast, other species excrete ingested DMA^V mainly unmetabolized, as a result of their toxicokinetics of DMA, which differs significantly from that of the rat. Studies of DMA metabolism in humans provide important information that when DMA is orally administered, DMA is excreted mostly unchanged. In human volunteers, 75% of a single oral dose of DMA^V was excreted in urine within 4 days, all as DMA^V; with no evidence of further methylation or demethylation (Buchet *et al.*, 1981). Marafante *et al.* (1987) reported that when a large dose of DMA (8 mg-As) was administered to a human volunteer, only 4% of the dose was excreted as TMAO and the rest as DMA^V. These results are very similar to findings of the mouse and hamster (two species refractory to DMA-induced bladder cytotoxicity and hyperplasia). Like humans, in hamsters and mice only about 5% of a DMA dose is excreted as TMAO (Marafante *et al*., 1987).

Rats retain more DMA in their red blood cells allowing for a long half life and enhanced metabolism compared to other species. As described in more detail in Appendix B, Lu *et al*. (2004) demonstrated that rats bind significantly more DMA^{III} to hemoglobin than humans. This finding is consistent with results from Shiobara *et al*. (2001), who examined the uptake of DMA^V and DMA^{III} into the red blood cells of rats, hamsters, mice, and humans. They found that DMA^{III} was taken up most efficiently in the rat cells and least efficiently in the human cells. Based on their results, the authors concluded that differences in uptake of DMA by red blood cells could contribute to differences in the reduction and methylation capacity between animal species (Shiobara *et al*., 2001).

In summary, the toxicokinetic feature of the rat, which allows for the generation of relatively large amounts of TMAO (and DMA^{III}) from the metabolism of ingested DMA, is in contrast to mice, hamsters, and humans, which excrete the DMA mostly unchanged without further metabolism. These metabolic differences translate into differences in the sensitivity to DMA-induced carcinogenicity because DMA^{III} accumulation in the bladder (in high enough amounts to cause increased cell proliferation) is the key rate-limiting step in the formation of DMA^V bladder tumors. Based on toxicokinetic differences between rats and humans, we believe the UF applied to the point of departure should be revised to reflect the unique sensitivity of the rat to DMA. This would require revising the UF currently recommended by EPA to <1 or 1 at the most.

5. Page 91, Section 6: Summary and Conclusions – Early life susceptibility

On page 91 of the Science Issue paper, the EPA concludes that a 10X FQPA safety factor "to protect children" should be included when developing the cancer reference dose for DMA. This decision is inconsistent with information presented earlier in the report, which points out that there is no indication that children would be more susceptible to bladder tumors that adults (p.71). This finding, in conjunction with data showing that there is no reproductive or developmental toxicity associated with DMA^V at doses that are not maternally toxic (LSRI, 1986; LSRI, 1988a; LSRI, 1988b), suggests that application of FQPA safety factor is unnecessary. Additionally, within EPA's regulatory framework for the evaluation of carcinogens, it is not appropriate to apply a 10X factor for early life susceptibility when there is no evidence of compound-related mutagenicity or developmental/reproductive adverse outcomes (US EPA, 2005b). Appendix B provides more detailed information regarding the developmental and reproductive effects of DMA^V, as well as EPA's guidance regarding the application of safety factors for early life susceptibility.

In summary, EPA's approach to develop a reference dose to evaluate DMA^V cancer risk is progressive and scientifically sound. However, some of the calculations and selection of the point of departure, as well as some of the UFs applied used to calculate a reference dose for DMA^V are not supported by the available scientific literature, much of which EPA cites in its own summary document. We urge the EPA to-re-examine the issues discussed above and refine its analysis to reflect the most scientifically supportable approach.

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Appendix A

Output from US EPA Benchmark Dose Software for the Hill Model for Regenerative Proliferation at 10 weeks from Arnold *et al.* (1999)

```
================================================================
         Hill Model. $Revision: 2.1 $ $Date: 2000/10/11 21:21:23 $
         Input Data File: C:\BMDS\DMA.(d)
         Gnuplot Plotting File:  C:\BMDS\DMA.plt
                                                  Mon Sep 19 10:53:36 2005
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = MEAN
   Independent variable = Dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = alpha * mean(i) ^ rho

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
                        alpha =     0.150474
                          rho =      1.43744
                    intercept =         0.22
                            v =         0.73
                            n =      4.14551
                            k =      5.76613

          Asymptotic Correlation Matrix of Parameter Estimates

                  alpha            rho     intercept               v               n
k

     alpha            1           0.79         0.074         -0.39      -0.00021      -
0.00045

       rho         0.79              1          0.34         -0.39      -0.00019      -
0.00074

 intercept        0.074           0.34             1          -0.33       0.00021
0.00081

         v        -0.39          -0.39         -0.33              1      -0.00089
0.0021

         n      -0.00021       -0.00019       0.00021       -0.00089             1
-1

         k      -0.00045       -0.00074       0.00081         0.0021            -1
1


                         Parameter Estimates

       Variable           Estimate            Std. Err.
          alpha           0.140747           0.0618874
            rho            1.40814            0.426711
      intercept           0.200807           0.0311312
              v            0.72786            0.101919
              n            11.9638             1054.38
              k            1.10562             9.78394


      Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	7	0.22	0.14	0.201	0.121	0.158
0.2	7	0.2	0.09	0.201	0.121	-0.00666
1	7	0.33	0.25	0.369	0.186	-0.21
4	7	0.95	0.42	0.929	0.356	0.0599
9.4	7	0.93	0.29	0.929	0.356	0.00374

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	31.699722	6	-51.399445
A2	40.257731	10	-60.515462
A3	38.643043	7	-63.286087
fitted	38.279551	6	-64.559103
R	12.356825	2	-20.713651

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	55.8018	8	<.0001
Test 2	17.116	4	0.001835
Test 3	3.22938	3	0.3576
Test 4	0.726984	1	0.3939

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems to adequately describe the data

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

```
              BMD =      0.920124

             BMDL =      0.425923


 Benchmark Dose Computation
Specified effect =          0.01

Risk Type        =     Extra risk

Confidence level =          0.95

              BMD =      0.753011

             BMDL =     0.0984536
```

Appendix B

Written Comments submitted to the Science Advisory Panel (SAB) on EPA's Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid and Recommendations for Dose Response Extrapolation

Comments on EPA's
Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid and Recommendations for Dose Response Extrapolation

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September 2, 2005

Table of Contents

Comments on EPA's
Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid and Recommendations for Dose Response Extrapolation

Executive Summary

Based on studies in rats, it is clear that the mode of action (MOA) for DMA^V-induced bladder tumors is cytotoxicity, resulting in persistent regenerative proliferation, leading to development of bladder tumors. This MOA has no mutagenic component and has a nonlinear dose response. The toxicokinetics of DMA^V is different from the toxicokinetics of DMA^V in other species, including humans, rendering the rat uniquely susceptible to DMA^V carcinogenicity.

In a recent report, *"Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid and Recommendations for Dose Response Extrapolation"*, the EPA Office of Pesticide Programs (OPP) uses a reference dose approach to estimate the daily exposure to DMA^V that is not associated with any increased cancer risk. The reference dose, 7×10^{-5} mg/kg/day is based on the $BMDL_1$ (*i.e.* the lower 95% confidence limit on the benchmark dose (BMD) that is associated with 1% of the maximum cell proliferation rate above background) and a composite Uncertainty Factor (UF) of 1000, composed of three 10-fold UFs - for intraspecies variability, interspecies variability, and the FQPA safety factor for special sensitivity of early life stages.

While OPP's framework is reasonable, several aspects of OPP's analysis are overly conservative and not based on the best available scientific data:

- The BMD_{10} rather than the BMD_1 is more appropriate as a point of departure (POD)
- The interspecies UF should be 1-fold at the most, since the model species, the rat, is more sensitive than humans to DMA^V.
- The FQPA UF should be 1-fold since there are data showing there is no increased sensitivity to DMA^V in early life stages compared to adults.

A more appropriate reference dose, based on sound scientific evidence, can be calculated as 2.9×10^{-2} mg/kg/day. This value is greater than that proposed by OPP, but is still protective of public health. When comparing this revised cancer reference dose to EPA's estimated daily intake of DMA^V due to agricultural use, the resulting margin of exposure (MOE) is over 100,000-fold.

1 Introduction

The Office of Pesticides Programs (OPP) of the United States Environmental Protection Agency (EPA, The Agency) recently issued, "*Science Issue Paper: Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation*" (US EPA, 2005a). In that document, the OPP provides a review of the toxicology of DMA^V, and an analysis of information from animal studies on the mode of action (MOA) for DMA^V-induced rat bladder tumors. OPP concluded that the MOA for DMA^V-induced rat bladder tumors involves cytotoxicity to the bladder epithelium, followed by a compensatory cell proliferation that leads to hyperplasia, and ultimately, a low incidence of bladder tumors (*i.e.*, the mode of action described by Cohen *et al*., 2002). The OPP concluded further that DMA^V-induced bladder tumors had a nonlinear dose response. In particular, the OPP analysis indicated that bladder tumor formation was a high-dose phenomenon that would occur only if the reactive compound dimethylarsinous acid (DMA^{III}) that is produced during the metabolism of DMA^V were generated in sufficient quantities to cause urothelial toxicity and a compensatory proliferative response.

Studies in rats have been used to characterize the MOA of DMA^V since the rat's toxicokinetic and toxicodynamic handling of this compound renders it the only species tested that is susceptible to DMA^V-induced bladder tumors. The rat is the only species known to generate enough of the reactive compound DMA^{III} to initiate the step-wise progression toward bladder tumors (Cohen *et al*., 2002). The dose of DMA^V required to cause rat bladder tumors is relatively high (approximately 8.0 mg/kg/day) (US EPA, 2001; Gur *et al*., 1989).

Based on this finding, the OPP recommended a "reference dose approach" to characterize DMA^V cancer risk in humans. In a process somewhat parallel to developing a reference dose (RfD) for noncancer endpoints, OPP identified a DMA^V dose from animal studies associated with negligible adverse effects. OPP then applied several uncertainty or safety factors to arrive at a daily dose of DMA^V that they estimated would be protective of public health. In developing a reference dose associated with DMA^V cancer risk, OPP made the following recommendations:

- Endpoint selection: Based on studies in rats that demonstrate the MOA for bladder tumors involves a statistically significant increase in cell proliferation induced by DMA^{III}, and that cell proliferation has a highly nonlinear dose-response, OPP recommended increased cell proliferation as the endpoint to conservatively model DMA^V cancer risk in humans.

- Benchmark dose analysis[3] and point of departure calculation: Relying on cell proliferation data in the bladder urothelium observed in rat studies, OPP recommended a benchmark dose (BMD) analysis to establish a BMDL[4]. The OPP recommended the $BMDL_1$ be used as a point of departure (POD).
- Uncertainty Factors (UF) application:
  - A UF of 10 was applied for intraspecies variability,
  - A UF of 10 was applied for interspecies variability,
- Food and Quality Protection Act (FQPA) safety factor application: OPP applied a 10-fold FQPA safety factor to account for the potential sensitivity of early life stages to DMA^V.

We have evaluated the scientific basis for OPP's methodology of using a "reference dose approach" to characterize the human health risk associated with DMA^V. Our analysis concludes OPP's decision to use a nonlinear approach (*i.e.*, reference dose approach) to describe DMA^V's dose-response relationship is supported; however, the choice of the specific POD, as well as two of the three uncertainty/safety factors, are not supported by the currently available scientific information. In this document, we provide a refined approach for developing the reference dose for DMA^V, based on the same MOA framework presented by EPA, but include changes to incorporate current species- and compound-specific data. Our recommendations result in a cancer reference dose for DMA^V that is higher than the value calculated by EPA, yet is still protective of public health.

2 Endpoint Selection

DMA^V, when fed to rats at sufficiently high doses, causes tumors in the bladder urothelium. The formation of these tumors progresses through a series of well defined events. Any of these events could be used as a suitable endpoint to derive a POD. Cancer risk assessments have typically used tumor formation as the endpoint. This choice is made when an MOA is not established and tumors remain the only measurable carcinogenic effect. Quantification of tumor response at low doses is associated with

[3] A benchmark dose (BMD) analysis is a more quantitative alternative for establishing a starting point (*i.e.*, POD) when evaluating the human health risks compared to the more conventional NOAEL/LOAEL approach. A BMD analysis uses a model to compute the amount of change associated with a specified adverse response considered to be biologically significant.

[4] BMDL refers to the corresponding lower limit of a one-sided 95% confidence interval on the BMD (*i.e.*, lower 95% confidence interval on the central estimate).

more uncertainty, and, thus, a tumor-based POD would require a larger margin of exposure to assure that the underlying process causing tumors is negligible at the POD.

Although tumor production and hyperplasia could both be used as endpoints for deriving the reference dose, OPP chose increased cell proliferation as "the rate limiting step for tumor formation" and correctly notes that "there must be a sufficient level of cytotoxicity and proliferation attained and sustained, to lead to hyperplasia and tumors". We agree with OPP that selection of cell proliferation is scientifically sound and health protective. From the non-tumor MOA endpoints, increased urothelial cell proliferation is the rate-limiting step and the key response that signals increased risk of tumors. Increases in cell proliferation can be quantified with a higher degree of confidence than increases in tumors. Proliferation shows a nonlinear dose response and a clear dose level below which cell division rates are unaffected. Doses that do not increase urothelial cell proliferation do not pose an increased bladder cancer risk. This endpoint is conservative since doses that cause increases in sustained cell proliferation do not necessarily cause increases in tumor incidence**.**

3 Benchmark Dose Analysis

A BMD analysis allows for fuller use of dose-response data. The agency has addressed statistical uncertainties in the calculation through the selection of a lower confidence limit on the BMD, *i.e.* the BMDL. However, by choosing the $BMDL_1$ instead of the $BMDL_{10}$ the OPP has introduced unnecessary uncertainty and conservatism into its analysis.

EPA's Draft Benchmark Dose Technical Guidance Document (US EPA, 2000) defines a BMD as “an exposure due to a dose of a substance associated with a specified low incidence of risk, generally in the range of 1% to 10%, of a health effect; or the dose associated with a specified measure or change of a biological effect". For continuous data, such as cell proliferation, the response can be dichotomized and treated as a quantal variable, or the magnitude in change can be expressed as a change in the mean response. Changes in mean response can be expressed in several ways, depending on the nature of the specific endpoint in question (US EPA, 2000). In the case of the DMA^V cell proliferation data, where there is a clear maximum response, OPP calculated the change in response to be a fraction of the range of responses because this endpoint had a clear maximum value. That is, OPP calculated the BMD_1 and BMD_{10} values based on a percent of the maximum value of cell proliferation above baseline.

For selection of the POD, the EPA recommends using the lower 95% bound on the BMD. Using the lower bound is a conservative measure that accounts for the uncertainty inherent in a given study, and assures that the specified change of response is not exceeded (US EPA, 2000).

OPP calculated a BMD_{10} and BMD_1 for cell proliferation using the data of Arnold *et al*. (1999), in which F344 rats were treated orally with DMA^V for up to 10 weeks to determine the effects on the bladder urothelium. Effects on cell proliferation were determined at 10 weeks. The specific values of 0.65 mg/kg/day for the BMD_{10} and 0.54 mg/kg/day for the BMD_1 were derived using the Hill model[5], the statistical model that best fit the experimental data. OPP chose to use the lower confidence interval on the BMD_1 (*i.e.* $BMDL_1$ [0.07]), which is approximately 115 times lower than the lowest dose that caused cancer in the two-year rat bioassay.

Several reasons support the use of the $BMDL_{10}$ rather than the $BMDL_1$:

1. The BMD_{10} and BMD_1 values are very similar (0.65 *vs.* 0.54). This shows that the dose-response curve for induced cell proliferation is very steep, with an almost step-like transition between doses that cause no increase and doses that cause a detectable and marked increase in cell proliferation. This feature increases the confidence that the low doses causing increases in cell proliferation have been identified. These low doses can be better be characterized by the more reliably estimated $BMDL_{10}$.

2. Cell proliferation at baseline is 0.20 ± 0.03 (standard error), while the cell proliferation at the BMD_{10} is 0.27[6]. The amount of excess cell proliferation at BMD_{10} is modest, only approximately 35% above controls and within the range of variability that occurs among controls.

3. There is substantially less uncertainty associated with the BMD_{10} than with the BMD_1. The greater uncertainty of the BMD_1 estimates is reflected in the relatively large confidence interval that surrounds this estimate compared to the BMD_{10}. The $BMD_{10}/BMDL_{10}$ ratio is 2.2, whereas the $BMD_1/BMDL_1$ ratio is 7.7.

4. Using the lower confidence limit on the BMD_{10} is conservative and appropriate for calculating a POD. In fact, the $BMDL_{10}$ is already 30 times lower than doses that lead to

[5] The Hill model determines a change in response that is a certain percent of the maximum amplitude (range between the control response and maximum response). The Hill model allows for non-linearity, contains an asymptote term for the estimation of a plateau, and is conservative because it does not allow a slope of zero, or a true threshold (US EPA, 2003). This model's ability to estimate a very steep dose-response curve appears to have been important in describing the Arnold *et al*., (1999) data.

[6] Based on the Hill model, the baseline (or control) cell proliferation rate was 0.20, and the maximal response was 0.93. The difference between the baseline and maximal response (i.e., 0.93-0.20), which is the maximum amplitude, was 0.73. Ten percent of the maximum change (0.73) is 0.07, so 10% of the maximum response is equivalent to 0.27 [i.e., 0.20 (baseline) + 0.07 (10% of the maximum amplitude). Similarly 1% of the maximum response over baseline is equivalent to 0.207 [i.e., 0.2 (baseline) + 0.007 (1% maximum amplitude)].

bladder tumors, and about 14 times lower than NOAEL for bladder tumors in the two-year bioassay.

4 Uncertainty Factors

4.1 Intraspecies Variability

The standard 10-fold uncertainty factor (UF) for variability in the human population is appropriate.

4.2 Interspecies

A UF of 10 is routinely used by EPA to account for interspecies variability, which includes both toxicokinetic and toxicodynamic components. According to EPA Food Quality Protection Act (FQPA):

> …for interspecies variability, a factor of 10-fold is applied as a default assumption to account for differences in sensitivity between species when animal data are used to assess human risk. Although the default 10X is generally used in the Agency, when data indicate that humans are less or more sensitive than animals, the interspecies group uncertainty factor of 10-fold may be reduced or raised (US EPA, 2002a).

In the case of DMA^V, using a UF of 10 is inconsistent with EPA guidance (US EPA, 2002a) based on data demonstrating that rats are substantially more sensitive to DMA^V toxicity than other animal species, including humans. Thus, for the reasons described below, the interspecies UF of 10 used by OPP to account for interspecies variability should be reduced to a value of 1 or less.

As described in OPP's analysis (US EPA, 2005a), the unique toxicokinetics of DMA^V in the rat, makes the rat particularly susceptible to DMA^V-induced cytotoxicity and tumorigenicity. Rat toxicokinetics of DMA^V differ from those of other species in a number of respects. First, the rat metabolizes DMA^V to trimethylarsenic oxide (TMAO) more extensively than do other species (Cohen et al., 2005), which indicates that more DMA^{III} is formed as a metabolic intermediate (US EPA, 2005a, p.32). The presence of DMA^{III} in sufficient quantities causes cytotoxicity in bladder cells, most likely through the interaction with sulfhydryl groups on macromolecules (Cohen *et al.*, 2005). As a consequence of the uniquely efficient metabolism of DMA^V in the rat, the rat is the only known species in which DMA^V administration results in urinary concentrations of DMA^{III} equivalent to those that are cytotoxic to urothelial cells *in vitro* and *in vivo* (Cohen *et al.*, 2002). In general, TMAO accounts for

roughly 40% of the urinary metabolites exposed to DMA^V (Yoshida *et al*., 1997; Yoshida *et al*., 1998; Cohen *et al*., 2002). This is in sharp contrast to mice and hamsters, which only excrete about 5% of a DMA^V dose as TMAO (Marafante *et al*., 1987). Human metabolism of DMA^V is much more like that of mice and hamsters, than that of the rat (Marafante *et al*., 1987).

Another feature that distinguishes rats from other species is the enhanced binding of arsenic compounds to rat hemoglobin (Lu *et al*., 2004). The increased binding compared to that of other species effectively prolongs the half-life of these compounds *in vivo* and may contribute to the high levels of TMAO generated in the rat. Lu *et al*. (2004) demonstrated that after incubation of red blood cells with graded concentrations of inorganic arsenic, at dose levels from 1 μM to 100 μM, the rat binds significantly more DMA^{III} to hemoglobin than humans. This enhanced binding may be involved in higher doses of DMA^{III} accumulating in the rat urothelium over time.

The available *in vitro* evidence suggests DMA^{III} induces comparable cytotoxicity in rat and human bladder cells. Cohen *et al*. (2002) demonstrated that the toxicity of DMA^{III} for the human bladder cell 1T1 (LC_{50}= 0.8 μM) was comparable to the toxicity for rat bladder cell line MYP3 (LC_{50}= 0.5 μM). More recently, based on differential gene expression determined with a microarray analysis, Sen *et al*., (2005) demonstrated that *in vitro,* gene expression changes were minimal in UROtsa cells (human bladder cells) after incubation with DMA^V. In contrast, MYP3 cells treated with DMA^V showed marked differential gene induction compared to controls. Because the toxicity in target cells appears to be comparable or less in humans compared to rats, and there is no indication of any additional sensitivity in humans, an interspecies UF to account for toxicodynamic differences is not necessary.

Thus, while the toxicity of DMA^{III} to urothelial cells is similar in rat and human cells *in vitro*, the quantity of DMA^{III} that accumulates in rat bladder *in vivo* is much higher than the amount that can form in a human bladder from a similar exposure. The extensive metabolism of DMA^V in the rat makes this species susceptible to DMA^{III}-induced cytotoxicity and ultimate tumor formation.

In summary, application of an interspecies UF greater than 1 is not warranted when there is sufficient evidence that the animal model is more sensitive than humans. Based on the overwhelming amount of data indicating that rats are *more* sensitive to DMA^V-induced bladder tumors, than any species, including humans, an UF of less than 1 would be appropriate to account for differential sensitivity between rats and humans, and an UF of 1 would still be sufficient for public health protection.

4.3 The FQPA Safety Factor

The "FQPA Factor" of 10 was designed to account for the uncertainty in the toxicity of noncancer endpoints, and was to be used if early life stages had not been fully tested for relevant noncancer endpoints (*i.e.* for neurodevelopmental toxicity, teratogenicity, *etc.*), and/or if early life stages show greater susceptibility to chemical-specific effects (US EPA. 2002b).

The issue of life-stage differences in responses to carcinogens is further discussed in EPA's recently revised guidelines "Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens" . These guidelines state:

> The Supplemental Guidance addresses a number of issues pertaining to cancer risks associated with early-life exposures generally, but provides specific guidance on potency adjustment only for carcinogens acting through a mutagenic mode of action….Default adjustment factors are meant to be used only when no chemical-specific data are available to assess directly cancer susceptibility from early-life exposure to a carcinogen acting through a mutagenic mode of action (US EPA, 2005b).

Thus, according to these recent guidelines, in the absence of evidence for early life special susceptibility, EPA recommends that no special adjustment be used for non-mutagenic carcinogens, or even for ones with uncertain modes of action. Only clearly mutagenic carcinogens that act *via* direct interaction with DNA warrant adjustment.

There is a large body of scientific literature demonstrating that DMA^V is not a direct acting mutagen and that any related genotoxicity is indirect (Cohen *et al*., 2005; Nesnow *et al*., 2002; Kligerman *et al*., 2003). Because there is clear evidence that DMA^V is not mutagenic and its indirect genotoxic effects are not integral to the MOA, an adjustment for early life sensitivity to DMA^V-induced carcinogenesis is not warranted.

General principles of bladder carcinogenesis would also argue against the application of the FQPA safety factor. As the EPA July 2005 document on DMA^V quite appropriately points out:

> The bladder and urinary tract are anatomically complete and functionally competent throughout life, which suggests qualitatively that there are no age dependent differences in susceptibility to chemically-induced cancer among humans. Furthermore, there is no indication that children are at any increased sensitivity, as bladder cancer is very uncommon at early ages in humans and given the late age of onset of bladder cancer, there is no evidence that, in general, there is a shortened latency for tumor development after childhood exposure (US EPA, 2005a).

Moreover, teratogenicity and multigenerational studies with DMA^V have demonstrated that the embryo is not especially sensitive to the effects of DMA^V toxicity, with any adverse developmental outcomes occurring only at maternally toxic doses (LSRI, 1986; LSRI, 1988a; LSRI, 1988b).

Thus, given that the lack of evidence of DMA^V-specific developmental toxicity at non-maternally toxic doses, the lack of DMA^{III} direct-acting mutagenicity, and the lack of evidence of increased susceptibility of children to bladder tumors, FQPA safety factor should be reduced to 1-fold.

5 Conclusions and Recommendations

The OPP has conducted a progressive and scientifically sound process to develop a DMA^V risk assessment by using a nonlinear "reference-dose approach". Their approach is consistent with the available data that indicate that DMA^V-induced rat bladder tumors occur only at relatively high doses and have a nonlinear dose response relationship . We agree with the OPP's use of a BMD approach and the use of cell proliferation data as the endpoint of concern. Using a BMD approach based on cell proliferation is adequately protective because it is the key, rate-limiting step process. Without increases in cell proliferation, no secondary cancer risk will occur. However, rather then the $BMDL_1$ as a POD (as OPP has suggested), we recommend the $BMDL_{10}$. The $BMDL_{10}$ will provide higher confidence that doses associated with negligible cell proliferation are appropriately characterized.

Several other aspects of OPP's analysis are based on default assumptions rather than on available, sound DMA^V-specific information on the species differences in toxicokinetics, toxicodynamics, and the relative susceptibility of early life stages. This information on DMA^V indicates that adjustments for interspecies differences and the FQPA (*i.e.*, early life susceptibility) are not necessary. Table 1 provides an overview of OPP's recommendations compared to alternative approaches that could be used to develop a cancer "reference dose" for DMA^V.

Using OPP's assumptions, the daily intake of DMA^V associated with negligible human cancer risk (*i.e.*, the cancer reference dose) would be is calculated as to be 7×10^{-5} mg/kg/day[7]. Using our recommendations for a POD and uncertainty/safety factors, the revised cancer "reference dose" for

[7] Calculated by applying a 10X UF for interspecies variability, a 10X UF for intraspecies variability, and a 10X FQPA safety factor to 0.07, which is the $BMDL_1$ associated with increased cell proliferation.

DMA^V would be 2.9 $x10^{-2}$ mg/kg/day[8]. This value is health protective, supported by the most current science and in accordance with most recent EPA guidance. While this analysis is focused on the carcinogenicity assessment for DMA^V, we note that estimated doses of DMA^V, based on EPA's HED evaluation (US EPA, 2001), are well below (*i.e.* more than 100,000-fold) the cancer reference dose noted above of 2.9 x 10^{-2} mg/kg/day.

Table 1.

An Alternate Approach Proposed for the Calculation of a Cancer "Reference Dose " for DMA^V

Input	OPP Choice	Gradient's Proposal	Comments
Dose-Response	Nonlinear	Nonlinear	OPP approach is appropriate. Studies in rats demonstrate the MOA for rat bladder tumors is cytotoxicity followed by regenerative hyperplasia. This MOA has a nonlinear dose-response relationship.
Endpoint	Cell Proliferation	Cell Proliferation	Cell proliferation is an early pre-cancer cursor and the rate-limiting event in DMA^V's MOA.
Point of departure	$BMDL_1$ (0.07 mg/kg/day)	$BMDL_{10}$ (0.29 mg/kg/day)	The $BMDL_{10}$ is associated with less statistical uncertainty, while still being conservative.
Intraspecies variability	10	10	The standard 10-fold interspecies UF is appropriate.
Interspecies UF	10	1	Toxicokinetic and toxicodynamic information indicates that the rat is more sensitive to DMA^V than humans.
FQPA Safety Factor	10	1	There is no indication of increased sensitivity of the young to DMA^V for noncancer endpoints and no indication that the young would be more susceptible than adults to bladder carcinogens.

In conclusion, while OPP's framework is reasonable, several aspects of OPP's analysis are overly conservative and not based on the best available scientific data:

- The BMD_{10} rather than the BMD_1 is more appropriate as a POD.
- The interspecies UF should be 1-fold at the most, since the model species, the rat, is more sensitive than humans to DMA.

[8] Calculated by applying a 1X UF for interspecies variability, a 10X UF for intraspecies variability, and a 1X FQPA safety factor to 0.29, which is the $BMDL_{10}$ associated with increased cell proliferation.

- The FQPA UF should be 1-fold since there are data showing there is no increased sensitivity to DMA^V in early life stages compared to adults.

Using a refined approach based on sound scientific principles, a more appropriate reference dose of 2.9×10^{-2} mg/kg/day can be calculated. This value is greater than that proposed by OPP, but is still protective of public health. When comparing this revised cancer reference dose to EPA's estimated daily intake of DMA^V due to agricultural use, the resulting MOE is over 100,000-fold.

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